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(54) Title: METHOD FOR BLEACHING WITH REDUCED ORGANIC CHLORIDES (57) Abstract A process for producing bleached lignocellulosic pulp for use in the pulp and paper industry having reduced total organi- cally bound chlorine and reduced brightness reversion. Lignocellulosic pulp is subjected to one or more xylanase treatment stages, in combination with one or more chemical bleaching stages.		

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"Method for bleaching with reduced
organic chlorides"

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TECHNICAL FIELD OF THE INVENTION

The present invention relates to a method
employing xylanases for reducing the level of
organically bound chlorine and reducing the brightness
reversion of chemically bleached lignocellulosic pulp
10 for use in the pulp and paper industry.

BACKGROUND OF THE INVENTION

The major components of lignocellulosic
materials (e.g. wood) are cellulose, hemicellulose, and
lignin. These compounds respectively comprise
15 approximately 35-50%, 20-30% and 20-30% of the dry
weight of woody plants. Cellulose is a saccharide
polymer composed of D-glucose units arranged in a
linear array. In woody plants, the linear cellulose
molecules are arranged in densely packed fibril
20 bundles. Hemicelluloses are linear and branched sac-
charide homo- and heteropolymers composed of various
five and six carbon sugars. These sugars include, for
example, xylose, arabinose, mannose, galactose and
glucose. Hemicelluloses consisting of a homopolymer of
25 one of these sugars would be termed, respectively,
xylan, arabinan, mannan, galactan and glucan.
Hemicelluloses participate in cross-linking the

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cellulose molecules and fibril bundles. The other major component of lignocellulosic material is lignin. Like cellulose and the hemicelluloses, lignin is a natural polymer. Lignin, however, is more complex than either cellulose or the hemicelluloses. It is composed primarily of methoxylated phenylpropane units which have been randomly linked by a variety of carbon-carbon and ether linkages, resulting in a three-dimensional matrix. This matrix encases the cellulose fibrils, imparting strength and rigidity to the composition. Thus, lignin can be considered as a kind of natural "cement" that holds together and surrounds the cellulose fibers.

Paper is basically a two-dimensional meshwork of randomly arranged cellulosic fibers linked by hydrogen bonds between the polysaccharide units. In woody plants the cellulose fibers are "cemented" into ordered parallel arrays by lignin. Therefore, to make woody plant material useful for papermaking, it must be separated into individual cellulosic fibers capable of hydrogen bonding to other cellulosic fibers. In conventional processing of woody plant material, fibers are liberated by mechanical grinding or refining, by chemical modification or removal of lignin, or by combinations of these methods. Fiber separation results in the formation of paper pulp -- a slurry or suspension of wood fibers. The deposition of these fibers into a tangled mat results in paper.

Mechanical pulping is the physical separation of woody fibers, producing so called high-yield pulp. High yield is obtained because lignin is not removed during the pulping process and so contributes its mass to the pulp.

In chemical pulping, the lignocellulosic material is treated with harsh chemical oxidants which

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degrade lignin. Chemical pulping is most commonly achieved with the Kraft (sulfate), sulfite, soda, and modified sulfite processes. These treatments remove the bulk of the lignin matrix, freeing the cellulose fibers and resulting in the formation of pulp. For example, the Kraft (sulfate) pulping process produces a pulp containing only 5-8% by weight of residual lignin, an approximately three to five fold reduction in the original lignin content.

Hybrid processes such as thermomechanical, chemithermomechanical and chemimechanical pulping have also found use.

Each of the above described pulping processes results in dark colored pulp, that color being due mostly to residual lignin. The intensity of pulp color depends on both the total amount of residual lignin and on its chemical state. For example, chemical pulps, from which most lignin has been removed during pulping, are especially dark in color because the remaining lignin is extensively oxidized and modified.

The residual lignin remaining in chemical pulps is particularly refractory to removal. This difficulty has been attributed to covalent bonding of residual lignin to hemicellulose (e.g., xylan), and perhaps to cellulose. Some of these bonds may be present in wood. However, the majority are thought to be formed during the chemical pulping process. See, e.g., Matsumoto et al., "The Role of Sugars Remaining in Residual Lignin", Fourth International Symposium on Wood and Pulping Chemistry, Paris, France, April 1987, Vol. 2, pp. 305-11; Iversen et al., "The Formation of Lignin-Carbohydrate Bonds During Kraft Pulping," Fourth International Symposium on Wood and Pulping Chemistry, Paris, France, April 1987, Vol. 2, pp. 163-65; Iversen and Wannstrom, "Lignin-Carbohydrate Bonds in a Residual

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Lignin Isolated from Pine Kraft Pulp", Holzforschung, 40, pp. 19-22 (1986).

There are many grades of paper, ranging from high quality white paper to that used in corrugated boxes. In order to produce a desirably bright paper product, the pulp must be brightened (bleached) prior to its conversion into paper.

The quality of brightness is commonly measured in terms of % G.E. - a measure of reflectance. The lignin content of pulp or paper products is commonly quantified in terms of kappa number. Although the dark color of pulp is due to lignin chromophores, brightness (% G.E.) is not directly proportional to lignin content (kappa number). For example, a bleaching stage which reduced the kappa number of a pulp from 25 to 15, without destroying lignin chromophores, would not significantly brighten the pulp. The kappa number must fall below about 8 to 12 before significant increases in % G.E. are observed as a result of lignin removal. Thus, extensive delignification may be required before measurable brightening is achieved.

Pulp bleaching is usually a multi-stage process. Brightening is not necessarily observed at each stage of a bleaching process, or even after the first several stages. It is the sum total of the bleaching stages that results in a brightened, bleached pulp. Single or plural stage processes which destroy lignin chromophores, remove lignin (delignify), or accomplish both these ends, might be termed bleaching stages.

The most commonly used chemical bleaching stages employ chlorine or chlorine-containing compounds such as chlorine dioxide, calcium hypochlorite or sodium hypochlorite. Chemical bleaching stages using

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hydrogen peroxide, oxygen and ozone have also found use. In commercial bleaching sequences, an alkaline extraction stage, followed by a water washing stage, would commonly follow each of the above chemical
5 bleaching stages in a particular bleaching sequence. Most commercial bleaching sequences comprise at least one chlorine or chlorine-containing compound stage. Chemical bleaching stages bleach lignocellulosic pulp primarily by removing its lignin, rather than by
10 destroying the lignin chromophores.

Lignocellulosic pulps that have been subjected to bleaching sequences comprising chlorine or chlorine-containing compound bleaching stages usually contain organically bound chlorine. And the paper
15 products produced from those pulps contain chlorinated organics. There is a growing demand for paper products with reduced levels of organically bound chlorine.

Bleaching sequences that do not comprise a chlorine or chlorine-containing compound treatment
20 stage produce bleached pulps with little or no organically bound chlorine. However, such non-chlorine chemical bleaching sequences have not been used widely because the bleaching stages they employ (e.g., oxygen, ozone or hydrogen peroxide stages) tend to be more
25 expensive than chlorine or chlorine-containing compound bleaching stages. In addition, certain of those stages tend to cause greater cellulose fiber degradation than chlorine or chlorine-containing compound bleaching stages. Such cellulose fiber degradation leads to low
30 viscosity pulp, which is productive of paper with poor mechanical properties.

For the foregoing reasons, most commercial bleaching sequences comprise at least one chemical bleaching stage that uses chlorine or a chlorine-
35 containing compound. Accordingly, a bleaching process

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that comprises at least one chlorine or chlorine-containing compound bleaching stage and that produces bleached lignocellulosic pulp with reduced total organically bound chlorine ("TOCl") is needed.

5 All paper products produced from lignocellulosic pulps that have been bleached by known bleaching sequences are subject to brightness reversion (i.e., yellowing) in response to heat, light and aging. Such brightness reversion is undesirable, especially in
10 high-quality white paper products. Accordingly, a commercially practical bleaching process that produces a lignocellulosic pulp with reduced brightness reversion is also needed.

Consistent with reports that residual lignin
15 of Kraft pulp is crosslinked to hemicellulose, several publications refer to treatment of wood pulp with hemicellulases (e.g., xylanase), sometimes in combination with chemical bleaching stages, for the purpose of delignification, brightening and/or
20 producing pulp that is productive of paper with improved mechanical properties. None of those publications suggests that xylanase treatment will reduce TOCl or reduce brightness reversion. What the publications do suggest is that hemicellulases, such as
25 xylanase, effect delignification by degrading hemicellulose, thereby rendering extractable the cleaved hemicellulose and any lignin that is crosslinked to it. See, e.g., Viikari I; Paice et al., "Bleaching Hardwood Kraft Pulp with Enzymes from Cloned Systems", Proceedings: 74th Annual Meeting of the
30 Canadian Pulp & Paper Association, Montreal, Canada, January 1988, pp. A133-36; Chauvet et al., "Assistance in the Bleaching of Never-Dried Pulps by the Use of Xylanases, Consequences on Pulp Properties", Fourth
35 International Symposium on Wood and Pulp Chemistry,

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Paris, France, April 1987, Vol. 2, pp. 325-27; Noé et al., "Action of Xylanases on Chemical Pulp Fibers", J. Wood Chem. Technol., 6, pp. 167-84 (1986); Viikari et al., "Bleaching with Enzymes", Proceedings: Third International Conference on Biotechnology in the Pulp and Paper Industry, Stockholm, Sweden, June 1986, pp. 67-69; French patent 2,557,894; United States patent 2,280,307.

SUMMARY OF THE INVENTION

10 The present invention relates, in one embodiment, to a practical method for producing bleached lignocellulosic pulp with reduced total organically bound chlorine ("TOCl") compared to that obtained using known bleaching sequences. In a second
15 embodiment, this invention relates to a method for producing bleached lignocellulosic pulp with reduced brightness reversion compared to that obtained using known bleaching sequences.

 Accordingly, it is an object of this
20 invention to provide a method for producing bleached lignocellulosic pulp that has a high viscosity and is productive of paper with superior mechanical properties, as well as having reduced TOCl and reduced brightness reversion.

25 These and other additional objects and advantages of the present invention, apparent from the detailed description and claims that follow, are accomplished in the first embodiment by subjecting lignocellulosic pulp to at least one xylanase treatment
30 stage, in combination with one or more chemical bleaching stages, wherein the chemical bleaching stage(s) comprise at least one stage utilizing chlorine or a chlorine-containing compound.

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In the second embodiment, the objects and advantages of the present invention are accomplished by subjecting lignocellulosic pulp to at least one xylanase treatment stage, in combination with at least one chemical bleaching stage.

DETAILED DESCRIPTION OF THE INVENTION

Lignocellulosic Pulps

Suitable lignocellulosic pulps for the practice of the present invention include pulps prepared from materials such as rice straw, wheat straw, bagasse, waste paper, hardwood and softwood. Hardwood and softwood pulps are preferred. By way of example, such wood pulps include those prepared by the well known sulfite, sulfate or kraft, soda and modified sulfite processes. Also suitable are mechanical pulps, thermomechanical pulps, and chemithermomechanical pulps. The foregoing is only a partial list of the lignocellulosic pulps that may be used to good advantage in the process of the present invention. For example, use of pulps produced by processes not presently known in the art is not excluded.

Xylanase Preparations

Xylanase preparations derived from either fungi or bacteria are useful for the purpose of this invention. Xylanase preparations comprising an endo-xylanase are preferred.

The particular microorganism used as a source of xylanase does not form a part of the present invention. Rather, any microorganism found to produce xylanases, and preferably endo-xylanases, is useful for the purpose of this invention. Many such microorganisms are known in the art. See, e.g., Ball and McCarthy, "Production and Properties of Xylanases

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from Actinomycetes", J. Appl. Bacteriol., 66,
pp. 439-44 (1989); Bastawde, "Studies on Xylanase from
Chainia Sp.", doctoral thesis (in biochemistry),
University of Poona, Division of Biochemical Sciences,
5 National Chemical Laboratory, Pune - 411008, India,
pp. 9-37 (May 1987) ("the Bastawde thesis"); Dekker,
"Biodegradation of the Hemicelluloses", in Biosynthesis
and Biodegradation of Wood Components, Academic Press,
NY, pp. 505-33 (1985).

10 Useful fungal species include those drawn
from, inter alia, Aspergillus, Chaetomium, Sporo-
trichum, Sclerotium, Schizophyllum, Trichoderma, and
Thermoascus. Particular fungal species believed to be
useful include, inter alia, Aspergillus ochraceus,
15 Aspergillus niger, Aspergillus awamori, Sporotrichum
dimorphosporum, Schizophyllum radiatum, Trichoderma
reesei, Trichoderma harzianum, and Thermoascus
aurantiacus.

Useful bacterial species include those drawn
20 from Chainia,* Streptomyces, Bacillus, and Clostridium.
Particular bacterial species believed to be useful
include, inter alia, Streptomyces olivochromogens,
Bacillus subtilis, Bacillus stearothermophilus,
Clostridium thermocellum, and Clostridium
25 acetobutylicum.

* It has been proposed that genus Chainia become a
junior synonym of the genus Streptomyces and that the
species it contains be renamed accordingly. Goodfellow
et al., "Transfer of Chainia Species to the Genus
30 Streptomyces with Emended Description of Species",
System. Appl. Microbiol., 8, pp. 55-60 (1986).
Approving this suggestion, the American Type Culture
Collection has reclassified the majority of its Chainia
strains to genus Streptomyces. The Northern Regional
35 Research Laboratory apparently disagrees with that
proposal; it has not reclassified its Chainia species.

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Chainia species, and those Streptomyces species formerly classified as being of the Chainia genus are preferred especially as sources of xylanase preparations.

5 Strains that produce xylanases, but not cellulases, are preferred.

Many strains from the genera and species recited above are available at public microorganism depositories. Useful strains (i.e., those that produce
10 xylanases) may be identified by the simple expedient of culturing the microorganisms, collecting the extracellular culture supernatant, and screening for xylanase activity according to procedures known in the art. See, e.g., Khan et al., "Assay of Xylanase and
15 Xylosidase Activities in Bacterial and Fungal Cultures", Enzyme Microb. Technol., 8, pp. 373-77 (1986).

We screened for xylanase activity sixteen strains of Streptomyces and Chainia obtained from the
20 American Type Culture Collection ("ATCC"). All but three were found to produce xylanases.

The lyophilized microorganisms obtained from the ATCC were cultured in the media recommended by the ATCC (ATCC Media #5; hereinafter "Sporulation Media")
25 (50 ml Sporulation Media in 250 ml Erlenmeyer flasks) at 26°C on a rotary shaker set at 150 rpm. After ample biomass had accumulated (approximately 2 weeks), a 10% (5 ml) inoculum was placed in 50 ml 3x Sporulation Media (contains all solutes of Sporulation Media at
30 three-fold higher concentration) in 250 ml Erlenmeyer flasks, and incubated for 7 days at 28-30°C on a rotary shaker set at 150 rpm. After the 7 day incubation, 10% inoculums (5 ml) from each culture were placed in 3% Xylan Fermentation Media (3% Larchwood Xylan (Sigma
35 Chemical Co. #X3875) and 1% yeast extract (Difco) in

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tap water) (Set A) and in 5% Wheat Bran Media (12.5% Post Natural Bran Flakes (40% wheat bran) and 1% yeast extract (Difco) in tap water) (Set B).

5 The cultures in 3% Xylan Fermentation Media (Set A) were incubated in 250 ml Erlenmeyer flasks at 30°C on a rotary shaker set at 220 rpm. Aliquots (3 ml) were removed from each Set A culture on each of days 3 to 8 after inoculation.

10 The cultures in 5% Wheat Bran Media (Set B) were incubated 4 days in 250 ml Erlenmeyer flasks at 28°C on a rotary shaker set at 150 rpm. On the fourth day of incubation, a 10% inoculum (5 ml) of each culture from Set B was aseptically transferred to 3% Xylan Fermentation Media and incubated in 250 ml
15 Erlenmeyer flasks at 30°C on a rotary shaker set at 220 rpm. Aliquots were removed from each Set B culture on each of days 3 to 8 after inoculation.

The aliquots were centrifuged at 70 x g_n for 20 minutes at room temperature and the supernatants
20 were assayed for xylanase activity as described infra. Note that since xylanase production peaks at different days in different strains, it is important to assay for activity on at least each of days 3 through 8 after inoculation.

25 Set B was incubated in the 5% Wheat Bran Media because that media has been said to induce xylanase production. See Srinivasan et al., "Studies on Xylan Degrading Enzymes from Chainia", Biotechnol. Lett., 6, pp. 715-18 (1984). We, however, observed no
30 significant difference in xylanase activity between the duplicate cultures of Set A and those of Set B.

Using each of the screening protocols described above, we identified ten strains as positive producers of xylanase. A strain was classified as a
35 positive producer if on any one of days 4 to 8 it was

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characterized by greater than 2 U/ml of xylanase activity. The ten positive producers (and their ATCC accession numbers) are: Streptomyces sclerotialis (ATCC 15896); Streptomyces flaviscleroticus (ATCC 19347);
5 Streptomyces fumigatiscleroticus (ATCC 19345);
Streptomyces minutiscleroticus (ATCC 17757); Streptomyces niger (ATCC 17756); Streptomyces ochraceiscleroticus (ATCC 15814); Streptomyces poonensis (ATCC 15723); Streptomyces roseiscleroticus (ATCC
10 17755); Streptomyces sp. (ATCC 27946); and Chainia hygroatrocyanea (ATCC 43962).

In addition to the ten positive producers recited above, we identified three strains as marginal producers of xylanases. A strain was classified as a
15 marginal producer if, on its highest-producing day of days 4 to 8, it was characterized by 1-2 U/ml of xylanase activity. The three marginal producers (and their ATCC accession numbers) are: Streptomyces olivaceiscleroticus (ATCC 15722 and 15897) and
20 Streptomyces purpurogeniscleroticus (ATCC 19348).

Only three of the sixteen strains screened were found to be negative producers. A strain was classified as a negative producer if, on its highest-producing day of days 4 to 8, it was characterized by
25 less than 1 U/ml of xylanase activity. The negative producers (and their ATCC accession numbers) are: Streptomyces ruber (ATCC 17754); Streptomyces violens (ATCC 15898); and Chainia yaxianensis (ATCC 43139).

Most preferred as a source of endo-xylanase
30 is Chainia sp. [NCL 82-5-1] (ATCC 53812). Xylanase II of that strain is preferred over Xylanase I. The hemicellulases of Chainia sp. [NCL 82-5-1] are discussed in the Bastawde thesis and in Srinivasan et al., "Studies on Xylan Degrading Enzymes from Chainia",
35 Biotechnol. Lett., 6, pp. 715-18 (1984). For further

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characterization of that strain see Srinivasan et al.,
"High Activity Extracellular Glucose (Xylose) Isomerase
from a Chainia Species", Biotechnol. Lett., 5, pp. 611-
14 (1983); Vartak et al., "Characterization of
5 Extracellular Substrate Specific Glucose and Xylose
Isomerases of Chainia", Biotechnol. Lett., 6, pp. 493-
94 (1984); and Pawar et al., "Purification and
Characterisation of Glucose (Xylose) Isomerase from
Chainia Sp. (NCL 82-5-1)", Biochem. Biophys. Res.
10 Commun., 155, pp. 411-17 (1988). An advantageous
feature of this Chainia strain is that it does not also
produce cellulases. Therefore, unfractionated
extracellular culture media, or concentrates thereof,
may be used without engendering the deleterious effects
15 of cellulases (i.e., reduced pulp viscosity and reduced
pulp yield).

Usually, the desired xylanases are secreted
by the microorganism into the extracellular culture
medium. The extracellular culture medium then is
20 harvested and processed to achieve the desired degree
of concentration and enzyme purity. The culturing con-
ditions and time of harvesting will influence the total
amount of enzymatic activity recovered as well as the
relative proportions of each of the various xylanases,
25 if more than one is present. In addition, various
strains differ in their total production of xylanases
and, if more than one xylanase is produced, in the
proportion of the various xylanases. Therefore, one
skilled in the art would optimize xylanase production
30 with respect to the desired final use of the enzyme
preparation. Methods for initiation and growth of the
fungal or microbial cultures and for the harvesting of
the extracellular growth medium for optimal xylanase
production do not form part of this invention and may

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conveniently be accomplished by methods known in the art.

5 The harvested culture media may be used directly. Preferably, though, the culture media is concentrated to produce an unfractionated xylanase concentrate for use in the process of the present invention.

10 The desirability of using extracellular growth media, or an unfractionated concentrate thereof, will be diminished when these preparations comprise significant quantities of active cellulases or proteases. Significant quantities of active cellulases would, by their attack on cellulose, cause undesirable decreases in pulp viscosity and, if enough were
15 present, measurably decrease pulp yield. Significant quantities of active proteases might, by their attack on other proteins, cause undesirable decreases in the activity of the xylanases in the reaction mixture. The undesirable effects of contaminating cellulases or
20 proteases may be diminished or avoided by adding inhibitors of these enzymes to the reaction mixture. Alternatively, the enzyme preparations may be fractionated to remove some or all of any contaminating cellulases or proteases.

25 Any concentration method that preserves enzymatic activity is appropriate. Suitable concentration methods include lyophilization and evaporation under vacuum, as well as precipitation with high salt, polyethylene glycol, acetone and alcohol.
30 If any of these concentration procedures is used, the xylanase may be reconstituted in water or in an appropriate buffer or pH-adjusted solution at pH 5-7, producing an unfractionated xylanase concentrate. Alternatively, the lyophilizate or precipitate may be
35 added directly to the pulp slurry.

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In some circumstances, it may be desirable to use partially purified subfractions of the harvested culture media. For example, use of a xylanase-containing subfraction that does not also contain significant quantities of cellulases or proteases is preferred. Alternatively, it may be desirable to separate various xylanases, so that these enzymatic activities may independently be used in enzyme treatments. Finally, we contemplate the use of xylanases that have been purified to near homogeneity, as well as use of mixtures of purified xylanases.

The methods used to subfractionate harvested extracellular culture media to achieve the desired degree of xylanase purification form no part of the present invention and may be accomplished by any effective combination of methods known to the art. For example, chromatographic separation steps based on differences in charge, pI, hydrophobicity or size may be employed. Also appropriate are various methods of selective precipitation, for example with ammonium sulfate. In addition, we anticipate the usefulness of affinity chromatography using, for example, monoclonal antibodies to purify selected xylanases. See, e.g., Tan et al., United States patent 4,725,544, which describes a process for separating xylanases from mixtures thereof with other hemicellulases and cellulases produced by culturing hemicellulytic micro-organisms.

Recombinant or synthetic xylanases would also be useful in the process of this invention. Such enzymes may be produced by methods known in the art.

Normally, the desired xylanase preparation is added directly to the pulp slurry. However, it may be advantageous to immobilize the enzymes on a solid support, and then to add this derivatized solid

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support. The procedure used to immobilize will depend upon the support used, and may be accomplished by any appropriate method which does not destroy enzymatic activity. An advantage to using xylanases coupled to solid supports is that the enzymes may, in some circumstances, be recovered more conveniently from the delignification reaction mixture after incubation with the pulp slurry. The recovered enzyme would then be reused.

10 Xylanase Treatment Stage

The xylanase treatment stage ("X stage") may be conducted in any container of the desired size for which, preferably, provision has been made for the mixing and temperature regulation of the contents. The order of addition of reaction components is not critical. The basic reaction mixture comprises lignocellulosic pulp and an active xylanase preparation, in water or an aqueous solution at the appropriate pH.

20 If the lignocellulosic pulp is a wood pulp, it should be present in the reaction mixture at a consistency of about 0.1 to 15%, and preferably at about 2 to 10%. A worker of skill in the art will, of course, routinely be able to determine the optimal pulp consistency for other lignocellulosic pulps.

25 The xylanase preparation is present in the reaction mixture at a ratio of about 0.1 to 200 units xylanase activity per gram dry weight pulp. Preferably, the xylanase preparation is present at about 0.1 to 50, and most preferably about 1 to 25, units xylanase activity per gram dry weight pulp.

30 One unit of xylanase activity is defined as that amount of enzyme which causes the production from xylan of one micromole of xylose per minute, under

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standard reaction conditions. Cleavage of xylan by xylanase produces reducing sugar moieties, which then are reacted with dinitrosalicylic acid ("DNSA") in the assay solution to produce a color change that is monitored at 540 nM.

A standard curve of xylose concentration (μ moles/ml) versus absorbance at 540 nM was generated using several dilutions of 100 mM xylose (Pfaltz and Bauer, Inc.) in 50 mM sodium acetate, pH 5.0. It was determined from the standard curve that one micromole of xylose has an absorbance at 540 nM of approximately 0.0128, under standard reaction conditions. This constant was used to calculate the xylanase activity of the sample solutions (e.g., culture supernatants) that were assayed.

The assay was linear for sample solutions with an absorbance at 540 nM of up to approximately 0.2. Sample solutions with absorbances above that value were diluted so that their absorbances were within the linear range.

For each sample solution (e.g., culture supernatant) assayed, a "reference solution" was prepared which contained the same amount of sample solution (e.g., culture supernatant), and enough 50 mM sodium acetate, pH 5.0 to give a final assay volume of 1 ml.

A solution of 1% larchwood xylan (Sigma Chemical Co. #X3875) in 50 mM sodium acetate, pH 5.0 (0.5 ml) was added to each sample tube. Next, a sufficient volume of 50 mM sodium acetate, pH 5.0 was added to the sample tubes to result in a final assay volume of 1 ml after the addition of sample solution (e.g., culture supernatant) and the xylan solution. Similarly, a sufficient volume of 50 mM sodium acetate was added to the reference tubes to result in a final

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assay volume of 1 ml after the addition of sample solution (e.g., culture supernatant). Next, the desired volume of sample solution (maximum volume, 0.5 ml) was added to each sample tube and reference tube.

5 The tube contents were mixed gently, and incubated at 50°C for 30 minutes. After incubation, 1% DNSA in distilled water (1 ml) was added to each tube and the tubes were incubated 5 minutes at room temperature. Then, 5 N NaOH (0.2 ml) was added to each tube (to
10 enhance color development) and the tubes were incubated for 30 minutes at room temperature. The absorbance at 540 nm of each sample solution then was measured, with the spectrophotometer blanked to zero on the sample's corresponding reference solution.

15 The pH of the reaction mixture is maintained within the range of about 4 to 8 throughout the incubation of the xylanase preparation with the lignocellulosic pulp. Preferably, the pH is maintained within the range of about 5 to 7.5. Usually, the pH of
20 the reaction mixture remains within the desired pH range. If, however, affirmative action is required to maintain the pH (e.g., where the pulp to be used has a particularly high or low pH), chemostatting may be employed. Chemostatting is the preferred method of pH
25 maintenance in large-scale incubations. Alternatively, the pH may be maintained (if maintenance be required), by use of a buffer in the reaction mixture. Any convenient buffer that is effective at the desired pH may be utilized, for example, phosphate. Buffer, when
30 added, is usually at a concentration of 0.1 to 100 mM in the reaction mixture.

The reaction mixture comprising the xylanase preparation and the lignocellulosic pulp is incubated at about 20 to 70°C and, preferably, at about 40 to
35 65°C. The incubation time is about 0.25 to 18 hours,

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preferably about 0.5 to 6 hours, and most preferably about 1 to 4 hours.

Preferably, the reaction components are mixed at the beginning of the incubation; further mixing is
5 not required.

Those of skill in the art readily will be able to optimize reaction conditions for the particular xylanase preparation utilized without undue experimentation.

10 If reuse of the xylanases in the reaction mixture is desired, they must be separated from the lignocellulosic pulp after the incubation. Appropriate methods to separate the xylanases from the pulp include vacuum filtration, precipitation and sedimentation.
15 Filtration is preferred.

Chemical Bleaching Stages

The choice and number of the particular chemical bleaching stage(s) used in combination with the X stage(s) does not constitute a part of this
20 invention. Rather, any sequence of chemical bleaching stages may be combined with one or more X stages according to the present invention, with the result that the bleached pulp so produced will have reduced TOC1 and reduced brightness reversion compared to pulp
25 subjected to comparable bleaching sequences, but without any X stages. Any type of chemical bleaching stage -- many of which are well known -- would be useful in the practice of this invention. Chemical
bleaching stages not now known in the art would also be
30 useful.

Moreover, the method of the present invention need not result in a fully bleached pulp. For example, a sequence according to this invention that produces a pulp with a brightness of only about 60% G.E. would

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still have lower TOCl than pulp subjected to the same bleaching sequence minus any X stages. As used herein, the term "bleached pulp" refers to a lignocellulosic pulp that has been subjected to at least one chemical
5 bleaching stage and at least one X stage and, as a result, is measurably delignified. Neither "full" bleaching nor brightening is required.

Commercial bleaching processes generally comprise a sequence of several types of chemical
10 bleaching stages. Examples of useful chemical bleaching stages that are well known include, inter alia, chlorine and chlorine dioxide (C_D), chlorine dioxide (D), and ozonation (Z). Generally, an alkaline extraction (E) stage is performed following each C_D , D
15 and Z stage. Such extraction stages may be enhanced with oxygen (E_O), hydrogen peroxide (E_P), or oxygen and hydrogen peroxide (E_{O+P}). Often, the pulp is washed with water between many of the above stages.

It should be understood that the above
20 recitation of chemical bleaching stages is presented by way of example only; it is not exhaustive. The objectives of this invention will be accomplished by combining one or more of any type of chemical bleaching stage with one or more xylanase treatment stages
25 according to this invention.

Treatment Sequences

The particular sequence of chemical bleaching stage(s) and X stage(s) does not form a part of the present invention. Rather, in the first embodiment of
30 the present invention, any sequence comprising at least one X stage and at least one chemical bleaching stage that utilizes chlorine or a chlorine-containing compound would produce a bleached pulp with reduced TOCl when compared to a pulp subjected to the same

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chemical bleaching stages, but not to any X stages. Similarly, in the second embodiment of the present invention, any sequence comprising at least one X stage and at least one chemical bleaching stage would produce
5 a bleached pulp with decreased brightness reversion when compared to a pulp subjected to the same chemical bleaching stages but not to any X stages.

Processes according to this invention that comprise one X stage are preferred.

10 The X stage(s) may be positioned to advantage at any point in the process sequence. For example, the X stage(s) may be the last stage(s) in the bleaching sequence. In a like manner, the chemical bleaching stages may be selected and combined in any order
15 desired. Examples of the many factors that those of skill in the art would consider routinely when choosing a particular sequence of stages according to this invention include, inter alia, the desired degree of bleaching, cost, and compatibility with existing bleach
20 plant equipment and processes. Preferably, however, the pulp will be washed with water immediately prior to any X stage.

In order that the present invention may be more fully understood, the following examples of the
25 process of this invention are set forth below. These examples are for purposes of illustration only and this invention should not be considered to be limited by any recitation used herein.

EXAMPLE 1

30 Xylanase Preparation from
Chainia Sp. [NCL 82-5-1]

Chainia sp. [NCL 82-5-1] (ATCC 53812) was maintained on potato dextrose agar slants at 4°C. The microorganisms were transferred into a sterile culture

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medium containing a xylan source (e.g. 5% wheat bran or 1% xylan) and conventional nutrients (e.g., 1% yeast extract). The cultures were incubated at 30°C in shaker flasks with vigorous shaking until peak xylanase activity (as measured by the assay described, supra) appeared (usually 3-5 days). After peak activity had been reached, the microbial cells and other solids were removed by conventional means (e.g., filtration or centrifugation) in order to obtain a clear filtrate or supernatant. The filtrate or supernatant typically was concentrated before use by ultra-filtration or by evaporation under vacuum. The yield of xylanase obtained from the cultures was approximately 10 U/ml if 5% wheat bran media was used, and approximately 25 U/ml if 1% xylan media was used.

EXAMPLES 2-7

Southern pine (softwood) kraft pulp was subjected to six different bleaching sequences, each consisting of an X stage in combination with several chemical bleaching stages.

The softwood kraft pulp used in Examples 2-7 was washed extensively with distilled water in a Buchner funnel and stored at 28% consistency at 4°C prior to use. The water-washed pulp had a kappa number of 34 and a viscosity of 33 cP.

In Examples 2-4, treatment sequences with "mock" X ("X_{MOCK}") stages served as controls. The X_{MOCK} stages were performed like the corresponding X stages, except that xylanase was not applied. In Examples 5-7, treatment sequences without either an X stage or an X_{MOCK} stage served as controls. The other stages of each control treatment sequence were performed as in the corresponding non-control sequence.

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After every treatment stage in each example, the pulp was collected by vacuum filtration in a Buchner funnel and then was washed in the funnel with distilled water (6 liters).

5 After each bleaching sequence, the brightness and total organically bound chlorine (TOCl) of the bleached pulp were measured. These results are displayed in Table I. Table I also displays the total available chlorine charge applied during the course of
10 each bleaching sequence.

To determine the TOCl of a pulp sample, pulp (5 g, dry weight basis) was extensively washed with deionized water in order to remove any residual inorganic and soluble organic chloride. The washed
15 pulp then was combusted in a closed flask according to the Schöniger method (see Kolthoff et al., Quantitative Chemical Analysis, p. 586 (4th ed. 1969). The chloride in the combusted sample was quantitated by ion chromatography (see Franklin and Fitchett, "Fast
20 Chemical Characterization of Pulping and Bleaching Process Liquors by Ion Chromatography", Pulp & Paper Canada, 83, pp. 40-44 (1982)).

To determine the brightness of a pulp sample, a pulp pad was made in accordance with TAPPI procedure
25 T218. The brightness (% G.E.) of the pulp pad then was measured according to TAPPI procedures T452 and T217 (TAPPI Test Methods, Vol. 1, TAPPI Press, Atlanta (1989)).

EXAMPLE 2: X_{MOCK}-C-E-D and XC-E-D Bleaching Sequences

30 The X stage was conducted as follows.
Chainia xylanase was prepared and dried by evaporation under vacuum as described in Example 1. A preparation of that xylanase (30,000 units dissolved in 1 liter of 50 mM sodium acetate, pH 5.0) was added to a slurry of

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200 g (dry weight basis) of water-washed pulp and 9 liters of sodium acetate, pH 5.0. The resulting pulp slurry, which was at a 2% pulp consistency, was incubated for 3 hours at 50°C, with continuous magnetic stirring. The X_{MOCK} stage was conducted essentially as described for the X stage, except that no xylanase was applied.

After the X stage, a portion of the treated, water-washed pulp (60 g, dry weight basis) was subjected to chlorination in a C_D stage. The pulp was placed in a polyester film bag (Scotch Pak #5, 3M Company). Then, chlorine and chlorine dioxide were added to give a 3% pulp consistency, 8.35% chlorine and 0.1% chlorine dioxide, with the percentages of chlorine and chlorine dioxide being based on dry weight pulp. The bag then was sealed, kneaded briefly until the reaction mixture became homogeneous, placed in a 45°C water bath, and incubated for 1 hour. The bag occasionally was removed from the water bath during the incubation and kneaded briefly to ensure proper mixing.

After the C_D stage, a portion of the treated, water-washed pulp (30 g, dry weight basis) was subjected to alkaline extraction in the presence of oxygen (E_O stage). The E_O stage was performed in a Parr reactor, with mixing at 320 rpm, at 70°C for 50 minutes using a 4% pulp consistency and 4% sodium hydroxide (on a dry weight pulp basis). Oxygen pressure was held at 40 psig for the first 20 minutes, and then at 30, 20, and 10 psig for the next consecutive 10 minute periods.

After the E_O stage, the treated, water-washed pulp (30 g, dry weight basis) was bleached with chlorine dioxide in a D stage. The D stage bleaching was performed in a polyester bag, with mixing provided by hand-kneading, as described above for the C_D stage. The reaction composition before incubation was as

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follows: 10% pulp consistency; 1% chlorine dioxide and 0.4% sodium hydroxide (based on dry weight pulp). The bag was incubated in a 70°C water bath for 3 hours. The pH of the pulp slurry at the end of the D stage was 3.0.

EXAMPLE 3: $X_{MOCK}C_DED$ and XC_DED Bleaching Sequences

In this example, the X, X_{MOCK} , C_D , and D stages were conducted essentially as described in Example 1. The E stage following the C_D stage was carried out in a polyester bag, as described above for the C_D stage, at 70°C for 1 hour, with a 10% pulp consistency and 4% sodium hydroxide (on a dry weight pulp basis).

EXAMPLE 4: $X_{MOCK}C_{D-O}ED$ and $XC_{D-O}ED$ Bleaching Sequences

In this example, the X, X_{MOCK} , E_O , and D stages were conducted essentially as described in Example 2. The C_D stage was conducted essentially as described in Example 2, except that less chlorine (6.8% chlorine and 0.1% chlorine dioxide based on dry weight pulp) was applied to the pulp (60 g, dry weight basis).

EXAMPLE 5: ZE_OD and XZE_OD Bleaching Sequences

In this example, the E_O and D stages were conducted essentially as described in Example 2. The X stage also was carried out as described in Example 2, except that less xylanase (10,000 units per 200 g dry weight pulp) was used.

After the X stage, the pulp slurry was filtered, and a portion of the treated pulp (100 g, dry weight basis) was reslurried at 2% pulp consistency with 0.1% diethylenetriaminepentaacetic acid (based on dry weight pulp), and dilute sulfuric acid was added to the slurry to lower the pH to 2.5. The pH-adjusted

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pulp slurry was stirred using a Lightning Mixer (1000 rpm) for 1 hour at 50°C, and the pulp then was recovered by vacuum filtration. The pulp was treated with dilute acid to remove metal ions, which are
5 thought to have an adverse effect on ozone bleaching.

To conduct the Z stage, the acid-washed pulp was slurried in water (1% pulp consistency) at pH 2.5 and placed in a closed reactor equipped with a mechanical mixer. Oxygen (100%) was passed through a
10 Welsbach Ozone Generator (model No. T408), which converted approximately 1.5-3.0% of the oxygen to ozone. This oxygen/ozone mixture then was bubbled into the bottom of the reactor through an inlet port at a flowrate of 2 liters/min. and a pressure of 6 psig. An
15 outlet port at the top of the reactor allowed exit of the oxygen/ozone mixture after its passage through the pulp slurry. The ozone concentration in the ozone/oxygen mixture at the inlet and at the outlet was measured with a Dasibi Ozone Monitor (Model No.
20 1003HC). Based on these measurements, it was determined that 2% ozone (based on dry weight pulp) was consumed during the reaction. The ozonation was carried out at room temperature for about 0.5 hour, with continuous mixing.

25 After the Z stage, a portion of the ozonated pulp (30 g, dry weight basis) was subjected to E₀D stages as described in Example 2.

In the control bleaching sequence (ZE₀D), no X stage was performed. The other stages were as
30 described immediately above.

EXAMPLE 6: ODED and OXDED Bleaching Sequences

The oxygen delignification (O stage) was carried out as follows. Water-washed pulp (290 g, dry weight basis) at a 10% pulp consistency was incubated

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with 3% sodium hydroxide and 0.5% magnesium sulfate (based on dry weight pulp) in a Quantum reactor, with mixing at 600 rpm (5 seconds every 3 minutes), under 80 psig oxygen pressure for 1 hour at 95°C. The kappa number of the resulting pulp was 18.72 after it had been washed with water as described above.

A portion of the oxygen-delignified, water-washed pulp (200 g, dry weight basis) was subjected to an X stage performed essentially as in Example 5. Next, a portion of the pulp (30 g, dry weight basis) was subjected to a first D stage that was performed essentially as described in Example 2, but with 1.78% chlorine dioxide (based on dry weight pulp). Following the first D stage, an E stage was conducted essentially as described in Example 3, but with 2.8% sodium hydroxide. Finally, a second D stage was performed essentially as described in Example 2, but with 1% chlorine dioxide (based on dry weight pulp). Both D stages and the E stage were conducted in polyester bags as described in Example 2.

In the control bleaching sequence (ODED), no X stage was performed. The other stages were as described immediately above.

EXAMPLE 7: C_DE_ODD and C_DXE_ODD Bleaching Sequences

Water-washed pulp (100 g, dry weight basis) first was subjected to chlorination in a C_D stage, which was conducted essentially as described in Example 2, except that 7.5% chlorine and 0.1% chlorine dioxide (based on dry weight pulp) were used.

The water-washed, chlorinated pulp was subjected to an X stage essentially as described in Example 5, except that half the xylanase (5,000 units) was used in order to keep the ratio of xylanase to dry weight pulp constant. A portion of the pulp (30 g, dry

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weight basis) next was subjected to an E_0 stage, performed essentially as described in Example 2. Finally, the pulp was subjected to two successive D stages, with a water wash between stages, using 1.5% and 0.5% chlorine dioxide (on dry weight of pulp basis), respectively.

In the control bleaching sequence ($C_D E_0 DD$), no X stage was performed. The other stages were as described immediately above.

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TABLE I

			Total Available Chlorine	Xylanase	Brightness	TOCl
5	<u>Example</u>	<u>Treatment Sequence</u>	<u>Charge</u>	<u>(U/g pulp)</u>	<u>(% G.E.)</u>	<u>(ppm)</u>
	2	X _{MOCK} C _D E _O D	11.2	0	86.1	300
		XC _D E _O D	11.2	150	88.1	232

	3	X _{MOCK} C _D ED	11.2	0	81.5	360
10		XC _D ED	11.2	150	86.1	258

	4	X _{MOCK} C _D E _O D	9.7	0	83.8	291
		XC _D E _O D	9.7	150	87.0	209

15	5	ZE _O D	2.6	0	85.0	95
		XZE _O D	2.6	50	89.1	36

	6	ODED	7.3	0	85.0	210
		OXDED	7.3	50	87.7	162
20	-----					
	7	C _D E _O DD	12.5	0	85.3	260
		C _D XE _O DD	12.5	50	85.8	212

25

EXAMPLES 8-10

Commercially obtained bleached hardwood and softwood kraft pulps were subjected to several different X stages.

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5 The pulps used in Examples 8-10 were washed extensively with distilled water in a Buchner funnel prior to use. They were stored at 4°C at a consistency, respectively, of 19.9% (softwood) and 12% (hardwood).

Control samples of the bleached hardwood and softwood kraft pulp were subjected to mock X stages performed under the same reaction conditions as the corresponding X stages, but without xylanase.

10 In Examples 8 and 9, the TOCl of each pulp was determined after the X or mock X stage according to the procedure described above for Examples 2-7.

15 In Examples 8-10, the brightnesses of the starting (i.e., pre-treatment) water-washed pulps and the pulps after xylanase or mock xylanase treatment were determined as described above for Examples 2-7. After that initial brightness determination, the pulp pads were subjected to two aging treatments intended to accelerate brightness reversion: (1) heating in an
20 oven at 105°C for 1 hour (Rapson and Spinner, "Brightnes Reversion in Bleached Pulp", in Singh, The Bleaching of Pulp, pp. 359-60 (TAPPI Press 1979); and (2) exposing to steam for 1 hour according to TAPPI procedure T260. The brightnesses of the pulps were
25 determined after these aging treatments.

Table II displays the results of the above analyses.

EXAMPLE 8: Xylanase Treatment of Bleached Softwood Pulp

30 A first sample of bleached softwood kraft pulp (50 g, dry weight basis) was slurried at 2% pulp consistency in 0.5 mM sodium acetate, pH 5.0. Chainia xylanase was prepared and dried by evaporation under vacuum as described in Example 1. A preparation of

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that xylanase (2,500 units dissolved in 250 ml of 50 mM sodium acetate, pH 5.0) was added to the pulp slurry.

5 A second sample of bleached softwood kraft pulp (50 g, dry weight basis) was slurried as the first sample was. A more concentrated Chainia xylanase preparation (7,500 units xylanase dissolved in 250 ml of 50 mM sodium acetate, pH 5.0) was added to this second pulp sample.

10 A third sample of bleached softwood kraft pulp (50 g, dry weight basis) also was slurried as the first sample was, but no xylanase was added. This sample served as a mock X control.

15 The above three pulp slurries were incubated for 2 hours at 50°C, with magnetic stirring using a Lightning Mixer. Then, the pulps were collected by vacuum filtration in Buchner funnels, and washed with distilled water (6 liters for each batch).

EXAMPLE 9: Xylanase Treatment of Bleached Hardwood Pulp

20 Xylanase treatments of bleached hardwood kraft pulp were performed essentially as described in Example 8.

EXAMPLE 10: Xylanase Treatment of Bleached Softwood Pulp

25 Bleached softwood kraft pulp (50 g, dry weight basis) was slurried in distilled water at 10% pulp consistency in a polyester bag. Xylanase (250 units) dissolved in 10 ml of distilled water was added to the pulp slurry, and the bag was sealed. The resulting
30 pulp slurry had a pH of 6.8. The slurry was kneaded in the sealed bag to mix and then incubated for 4 hours in a 60°C water bath. The bag occasionally was removed from the water bath and kneaded briefly to mix. A control pulp sample was incubated without xylanase.

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After the incubation, the pulps were washed with distilled water (6 liters), as described above.

TABLE II

	<u>Example</u>	<u>Pulp</u>	<u>Xylanase (U/g pulp)</u>	<u>TOCl (ppm)</u>	<u>Initial Bright- ness (% G.E.)</u>	<u>Brightness (%G.E.) After Aging Treatment:</u>	
						<u>Oven</u>	<u>Steam</u>
5							
10	8	SWD	0 (starting)	ND	87.9	86.9	82.3
			0 (mock X)	245	88.0	86.0	83.2
			50	195	88.7	86.5	85.1
			150	200	87.3	85.9	85.7
15	9	HWD	0 (starting)	ND	84.2	81.5	78.1
			0 (mock X)	590	84.9	81.1	78.3
			50	490	86.1	83.6	81.3
			150	480	86.1	84.9	82.4
	10	SWD	0 (mock X)	ND	87.7	84.0	80.0
			5	ND	88.4	84.9	82.0

20 * "SWD" is bleached softwood kraft pulp; "HWD" is bleached hardwood kraft pulp.

** Not determined.

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We claim:

1. A method for producing bleached lignocellulosic pulp with reduced total organically bound chlorine comprising:

(a) one or more xylanase treatment stages comprising the step of incubating lignocellulosic pulp with an effective amount of a xylanase preparation; and

(b) one or more chemical bleaching stages, wherein at least one chemical bleaching stage employs a member selected from the group consisting of chlorine, chlorine-containing compounds and mixtures thereof.

2. A method for producing bleached lignocellulosic pulp with reduced brightness reversion comprising:

(a) one or more xylanase treatment stages comprising the step of incubating lignocellulosic pulp with an effective amount of a xylanase preparation, and

(b) one or more chemical bleaching stages.

3. The method according to either of claims 1 or 2, wherein the lignocellulosic pulp is selected from pulps of rice straw, wheat straw, bagasse, waste paper, hardwood and softwood.

4. The method according to claim 3, wherein the lignocellulosic pulp is selected from hardwood kraft pulp and softwood kraft pulp.

5. The method according to either of claims 1 or 2, wherein the xylanase preparation is derived from a microorganism selected from the group consisting of strains of Aspergillus, Sporotrichum, Sclerotium,

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Chaetomium, Schizophyllum, Chainia, Clostridium,
Streptomyces, Bacillus and Trichoderma.

6. The method according to claim 5, wherein the xylanase preparation is derived from a strain of Chainia or Streptomyces.

7. The method according to claim 6, wherein the strain is Chainia sp. [NCL 82-5-1], having the identifying characteristics of ATCC 53812.

8. The method according to claim 6, wherein the strain is selected from the group consisting of: Streptomyces sclerotialus, having the identifying characteristics of ATCC 15896; Streptomyces flaviscleroticus, having the identifying characteristics of ATCC 19347; Streptomyces fumi-gatiscleroticus, having the identifying characteristics of ATCC 19345; Streptomyces minutiscleroticus, having the identifying characteristics of ATCC 17757; Streptomyces niger, having the identifying characteristics of ATCC 17756; Streptomyces ochraceiscleroticus, having the identifying characteristics of ATCC 15814; Streptomyces poonensis, having the identifying characteristics of ATCC 15723; Streptomyces roseiscleroticus, having the identifying characteristics of ATCC 17755; Streptomyces sp., having the identifying characteristics of ATCC 27946; and Chainia hygroatrocyanea, having the identifying characteristics of ATCC 43962.

9. The method according to either of claims 1 or 2, wherein there is one xylanase treatment stage.

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10. The method according to claim 9, wherein the xylanase treatment stage is the last stage.

AMENDED CLAIMS

[received by the International Bureau on 4 April 1991 (04.04.91)
original claims 1,2,9 and 10 amended; other claims
unchanged (3 pages)]

We claim:

1. A method for producing bleached lignocellulosic pulp that is characterized by total organically bound chlorine below a preselected level comprising:

(a) one or more xylanase treatment stages comprising the step of incubating lignocellulosic pulp with an effective amount of a xylanase preparation;

(b) one or more chemical bleaching stages, wherein at least one chemical bleaching stage employs a member selected from the group consisting of chlorine, chlorine-containing compounds and mixtures thereof;

(c) measuring the total organically bound chlorine at some point after step (a) to determine whether it is below the preselected level; and

(d) repeating step (a) if the total organically bound chlorine measured in step (c) is above the preselected level.

2. A method for producing bleached lignocellulosic pulp that is characterized by brightness reversion below a preselected level comprising:

(a) one or more xylanase treatment stages comprising the step of incubating lignocellulosic pulp with an effective amount of a xylanase preparation;

(b) one or more chemical bleaching stages;

(c) measuring the brightness reversion at some point after step (a) to determine whether it is below the preselected level; and

(d) repeating step (a) if the brightness reversion measured in step (c) is above the preselected level.

3. The method according to either of claims 1 or 2, wherein the lignocellulosic pulp is selected from pulps of rice straw, wheat straw, bagasse, waste paper, hardwood and softwood.

4. The method according to claim 3, wherein the lignocellulosic pulp is selected from hardwood kraft pulp and softwood kraft pulp.

5. The method according to either of claims 1 or 2, wherein the xylanase preparation is derived from a microorganism selected from the group consisting of strains of Aspergillus, Sporotrichum, Sclerotium, Chaetomium, Schizophyllum, Chainia, Clostridium, Streptomyces, Bacillus and Trichoderma.

6. The method according to claim 5, wherein the xylanase preparation is derived from a strain of Chainia or Streptomyces.

7. The method according to claim 6, wherein the strain is Chainia sp. [NCL 82-5-1], having the identifying characteristics of ATCC 53812.

8. The method according to claim 6, wherein the strain is selected from the group consisting of: Streptomyces sclerotialis, having the identifying characteristics of ATCC 15896; Streptomyces flaviscleroticus, having the identifying characteristics of ATCC 19347; Streptomyces fumi-gatiscleroticus, having the identifying characteristics

of ATCC 19345; Streptomyces minutiscleroticus, having the identifying characteristics of ATCC 17757; Streptomyces niger, having the identifying characteristics of ATCC 17756; Streptomyces ochraceiscleroticus, having the identifying characteristics of ATCC 15814; Streptomyces poonensis, having the identifying characteristics of ATCC 15723; Streptomyces roseiscleroticus, having the identifying characteristics of ATCC 17755; Streptomyces sp., having the identifying characteristics of ATCC 27946; and Chainia hygroatrocyanea, having the identifying characteristics of ATCC 43962.

9. The method according to either of claims 1 or 2, wherein there is one xylanase treatment stage in step (a).

10. The method according to claim 9, wherein the xylanase treatment stage of step (a) is after the one or more chemical bleaching stages of step (b).

STATEMENT UNDER ARTICLE 19

Applicant has amended claims 1, 2, 9 and 10 (pages 33-35) as set forth above to claim its invention with more particularity. Applicant encloses substitute pages 33-35 having these amendments.

Applicant has amended claims 1 and 2 -- the only independent claims -- to make clear that its invention is processes for producing bleached lignocellulosic pulp characterized by total organically bound chlorine (claim 1) or brightness reversion (claim 2) below a preselected level. Accordingly, applicant has amended the claims to recite two additional process steps. Added step (c) requires measuring the total organically bound chlorine (claim 1) or brightness reversion (claim 2) of the lignocellulosic material at some point after step (a). Added step (d) requires repeating step (a) if the measured total organically bound chlorine (claim 1) or brightness reversion (claim 2) is above the preselected level. Claims 9 and 10 have been amended to be consistent with amended claims 1 and 2.

Support for the claim amendments made herein is apparent from, and inherent in, the specification. See, e.g., Examples 2-10.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05933

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ¹ According to International Patent Classification (IPC) or to both National Classification and IPC IPC (5) : D21C 9/10 U.S. CL.: 162/72; 435/277,278		
II. FIELDS SEARCHED <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin: 5px 0;">Minimum Documentation Searched ²</div> <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;">Classification System</div> <div style="width: 55%;">Classification Symbols</div> </div> <div style="margin-top: 10px;"> U.S. 162/1,72 435/277,278 </div> <div style="text-align: center; border-top: 1px solid black; border-bottom: 1px solid black; margin-top: 10px;">Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched ³</div>		
III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁴		
Category ⁵	Citation of Document, ⁶ with indication, where appropriate, of the relevant passages ⁷	Relevant to Claim No. ⁸
Y	Biotechnology in the pulp and paper industry, The third international conference June 16-19 1986. STOCKHOLM, (VIAKARI ET AL); (" Bleaching with Enzymes", pages 67-69. See page 69.	1-10
Y	Fourth international symposium on wood and pulping chemistry, April 27-30 1987, "PARIS assistance in bleaching of never-dried pulps--", pages 325-327 See abstract.	1-10
Y	Arch. Microbiology, Vol 112, 1977. (LUNDQUIST ET AL), " Fungal Degradation of kraft Lignin and Lignin Sulfonates prepared from Synthetic C 14-Lignins" pages 291-296, See pages 292 and 196.	10
<div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>⁹ Special categories of cited documents: ¹⁰</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 55%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step</p> <p>"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
IV. CERTIFICATION		
Date of the Actual Completion of the International Search ¹¹		Date of Mailing of this International Search Report ¹²
07 DECEMBER 1990		04 FEB 1991
International Searching Authority ¹³		Signature of Authorized Officer ¹⁴
ISA/US		 STEVE ALVO